Xdrop® DE20 and DE50 droplet production is compatible with common growth media

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Summary

- Xdrop double-emulsion droplets are compatible with a broad range of media used for growing microbial and mammalian cells.

Introduction

Droplet microfluidics has revolutionized the study of single cells. Using Xdrop's picoliter double-emulsion droplets (DE20 and DE50 droplets) to isolate living cells facilitates the extraction of a wide range of insights, including single-cell functionality.

Xdrop technology only requires low input volumes, making large screening efforts fast and affordable. Furthermore, the double-emulsion droplets (Figure 1) are compatible with droplet- and cell-sorting technology. The oil used to produce the droplets contains no components of human or animal origin, ensuring their suitability for animal cell work.

The Xdrop encapsulation process creates an isolated compartment for a single cell's immediate environment. The high-throughput workflow supports the analysis of thousands to millions of single cells, making it suitable for heterogeneous population analysis and rare event discovery. For such applications, the droplets must be compatible with common growth media.

Here, we report on the compataibility of the Xdrop double-emulsion droplet production process with various media for growing microbial or mammalian cells as well as some other common media used in cell work.

Methods

All media and solutions listed in Table 1 were tested using an Xdrop and Xdrop DE20 or DE50 Cartridges and Gaskets. The inner phases for the double-emulsion droplets were prepared with or without supplements and sterile filtered. Droplets were produced by loading the cartridges in the Xdrop instrument and selecting the DE20 or DE50 program for droplet generation.

Results

DE20 and DE50 droplet production was generally successful when media for bacteria, yeast, or mammalian cells were used. For 18 out of the 19 media tested, the success rate was over 95% and for the remaining medium, the success rate was over 80% (Table 1). Surprisingly, droplet production using unfiltered milk was successful, despite the particle content that could potentially have blocked the small channels of the cartridges. Figure 2 shows microscope images of droplets in three media.

Note: We have successfully encapsulated *Escherichia coli*, *Saccharomyces cerevisiae*, and human natural killer (NK-92), Ramos B, lymphoblast (K562), and embryonic kidney 293 (HEK-293) cells using Xdrop (data available on request).

Conclusion

The high success rate demonstrates that the Xdrop double-emulsion droplet system has the potential to encapsulate living bacterial, yeast or mammalian cells with a wide variety of growth media. Cell growth potential is further supported by the possibility to replace the outer phase with growth media, including supplements, and the partial permeability of the oil layers of the double-emulsion shell, allowing for nutrient and gas exchange.

Learn more about Xdrop at samplix.com.

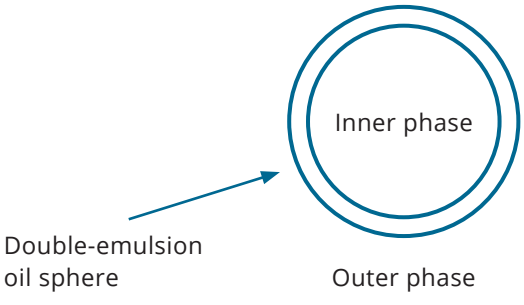
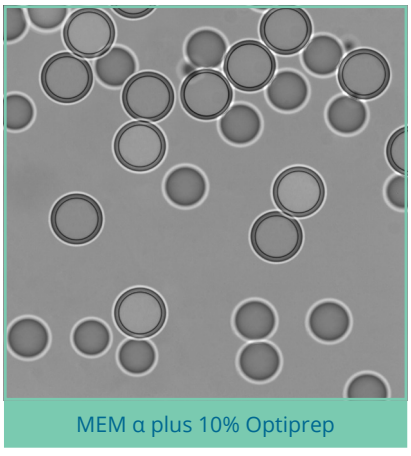


Figure 1. Xdrop double-emulsion droplet structure. They are composed of an oil sphere filled and surrounded by aqueous solutions (referred to as a water-in-oil-in-water system). The oil contains no components of animal origin, making the droplets suitable for animal cell analyses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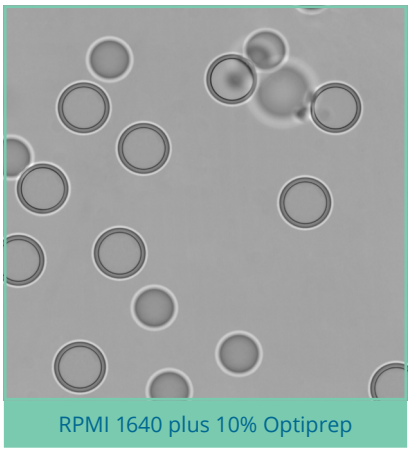
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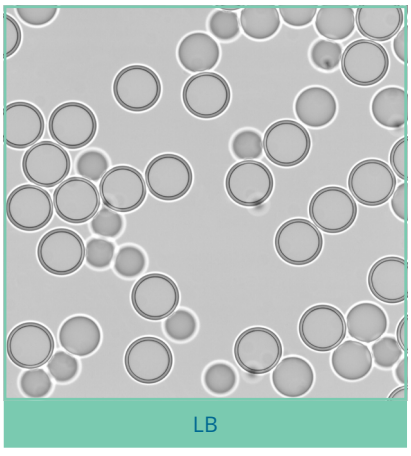
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Figure 2. Microscope images (magnification 20x) of DE50 droplets produced with three of the tested media. In all three cases, the outer phase is the same as the growth medium with the addition of Samplix Stabilizing Solution for cells.

Table 1. Xdrop double-emulsion droplet production in various growth media.

Based on the number of successful droplet productions compared to the number of tests, the productions are coded green (100% success rate); turquoise (>90% success rate); or grey (80–90%, some additional validation may be required). In the cartridge column, for ease of reading, blue shading indicates DE20 Cartridges; no shading indicates DE50 Cartridges. SS = Samplix Stabilizing Solution. SSfc = Samplix Stablizing Solution for cells

Application	Inner phase	Outer phase	Cartridge	Success
For bacterial cells	LB: Lysogeny broth	Inner + SSfc	DE20	100%
	M2: Basic culture media	Inner + SS	DE20	100%
	M-17	Inner + SS	DE20	100%
	MRS: De Man, Rogosa and Sharp	Inner + SS	DE20	100%
	LB	Inner + SSfc	DE50	100%
	M9	Inner + SSfc	DE50	100%
For mammalian cells	RPMI 1640: Roswell Park Memorial Institute 1640 solution	Inner + SS	DE20	100%
	RPMI 1640 with 10% FBS	Inner + SS	DE20	>80%: some additional validation may be needed
	DMEM: Dulbecco's modified Eagle's medium	Inner + SS	DE20	100%
	DMEM with 10% FBS	Inner + SS	DE20	100%
	Eagle's MEM: Eagle's minimal essential medium	Inner + SS	DE20	100%
	Eagle's MEM with 10% FBS	Inner + SS	DE20	100%
	MEM α with 10% OptiPrep™, 12.5% FBS, and 12.5% horse serum	Inner + SSfc	DE50	97%
	RPMI 1640 with 10% OptiPrep and 10% FBS	Inner + SSfc	DE50	98%
	DMEM with 10% OptiPrep and 10% FBS	Inner + SSfc	DE50	100%
For yeast	YPD: Yeast extract, peptone, and dextrose broth	Inner + SSfc	DE20	100%
	YPD	Inner + SSfc	DE50	100%
	YNP: Yeast nitrogen base	Inner + SSfc	DE50	100%
Misc.	Unfiltered skimmed milk	Inner + SS	DE20	100%